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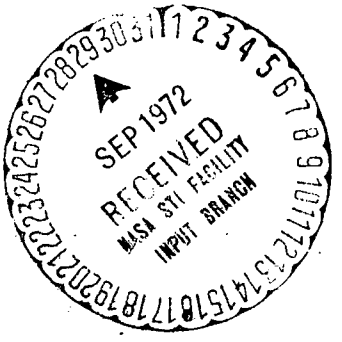

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THE TERMINAL DECONTAMINATION OF ROOMS. EVALUATION OF EFFICACITY CHECK

G. Reybrouck and H. van de Voorde*

ABSTRACT. The authors describe the various methods successively applied to check the efficacity of the terminal decontamination of rooms by means of gaseous formaldehyde.

The following method has been accepted: ten penicylinders and ten microscope slides are contaminated by a dense bacterial suspension of St. aureus; a similar series is inoculated by a diluted suspension. For E. coli, Ps. aeruginosa and the spores of Bacillus subtilis only one penicylinder and one slide are exposed.

All the carriers were exposed to the action of the formaldehyde vapor in the test room. Although a high level of formaldehyde in the air was measured, not all the carriers inoculated by a high inoculum of bacteria were sterilized. The causes of the failure are discussed, and recommendations for further investigations are formulated.

I. Introduction

Of all the gaseous disinfectants, formic aldehyde, or formol, or methanal is the most frequently employed. /199**

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** Numbers in the margin indicate pagination in the original foreign text.

Trillat discovered its antiseptic properties in 1888. This colorless gas possesses a special, extremely irritating odor. It is water-soluble.

Depending on the conditions, formaldehyde polymerizes very easily to yield paraformaldehyde, a white, water-insoluble mass, or trioxymethylene which, upon volatilization, again yields formaldehyde. The combustion of trioxymethylene is often used to generate formol.

Formaldehyde reacts with ammonia to form hexamethylenetetramine (used in medicine under the name urotropin). One takes advantage of this property to neutralize formol after the disinfection (...).

"Formol vapors have a density essentially equal to that of air and are very diffusible, which explains the instructions to hermetically seal the rooms to be disinfected using formol. Formol vapors possess a most remarkable germicidal ability. Trillat, Berlioz and Trillat, Philip, and Miquel exposed most of the pathogenic bacteria to the action of this gas and found that, with the exception of spores, all were killed in varying lengths of time. Investigations have been conducted in chambers measuring more than 100 m^3, with the intention of applying this powerful germicide to the decontamination of rooms. The results have been conclusive, provided that the room was saturated with formol vapors.

"It has been found that, under these conditions, most of the formaldehyde immediately condenses on the wall surfaces to the extent that the antiseptic action is attributed not to the gas, but to the formaldehyde solution resulting from the condensation of the former on the surfaces of objects.

"It was concluded from this that the atmosphere of the room to be disinfected had to be charged with water vapor.

"Investigators failed in disinfection tests, the adjunction of water vapor notwithstanding, and formol is regarded as a surface disinfectant which should be reserved exclusively for the disinfection of rooms."

This text appears on pages 512 and 513 of Précis d'Hygiène by Jules Courmont, Professor of Hygiene at the Faculty of Medicine at Lyon (Courmont, J., Précis d'Hygiène, Masson et Cie., Publishers, 1914).

The object of this paper is a critical approach to the method classically employed in Belgium to evaluate the effectiveness of the terminal decontamination of rooms. Such an evaluation must take into account the species of the supposedly representative microorganisms, inoculum size, exposure time, the type and number of carriers to be exposed, the time and expense necessary to successfully complete this evaluation. /200

Another approach to the problem is to determine the concentration of disinfectant gas required to guarantee adequate disinfection. Then the latter could be evaluated by simply measuring the quantity of disinfectant in the air.

2. Methodology

Over the years, different methods have been applied in the laboratory to evaluate the effectiveness of the terminal decontamination of a room.

2.1. Initially, we were content to use the method involving Petri dishes containing nutrient media inoculated with suspensions of bacteria. The Petri dishes were placed in the center of the test room, and in a corner as well. The first finding concerned the importance of the number of bacteria inoculated; a small number was more easily sterilized than a larger inoculum. In a test, for example, an inoculum of 23,000 staphylococci does not seem to be influenced by formolization, whereas no organisms developed from an inoculum bearing 23 staphylococci.

2.2. Later, A.O.A.C. directives [2] led us to employ microcylinders in addition to Petri dishes. Since the literature [7, 8] indicates that formol reaches the interior of objects with difficulty, it seemed that this control method would be more severe than the Petri dish method. We adopted the following procedure. Sterile, metal microcylinders are placed in a saline suspension of bacteria (an 18-hour growth on Trypticase Soy Agar, BD-Merieux). These cylinders are removed after 15 minutes, placed on filter paper in a Petri dish, then dried at 37° C for thirty minutes. They are exposed to formol in this dry state. At the end of the disinfection test, these microcylinders are placed in a nutrient broth (Tryptic Soy Broth, Difco) and incubated at 37° C for 48 hours. It should be noted that this method furnishes only qualitative data. A typical result of such a test appears in Table I: the Petri dishes seem to be sterilized, but the microcylinders are not.

2.3. These data caused us to question the value of the method using Petri dishes and to conduct the following tests. We poured different solid nutrient media into a series of Petri dishes: Trypticase Soy Agar (TSA) (BD-Merieux), Nutrient Agar (NA) (Oxoid), Plate Count Agar (PCA) (Difco), Tryptone Glucose Yeast Extract Agar (TGYA).

TABLE I

Species of microorganisms	Bacterial count	Growth			
		Petri dishes		Cylinders	
		Middle	Corner	Middle	Corner
St. aureus	$1.7 \cdot 10^7$	-	-	+	+
E. coli	$2.6 \cdot 10^7$	-	-	-	+

Evaluation of the terminal decontamination: warm nebulization of formalin 7 ml/m^3

(Exposure time: 6 hours; temperature: 29° C; relative humidity: 82%)

Two other series of dishes contained these same media as well as histidine (final concentration of 1 mg/ml) and sodium sulfite (60 mg/ml), respectively.

Three dishes were made per medium and per additive. The first and second dishes were inoculated with the bacterial suspension (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa) — the first serving as a control, the second undergoing formolization at the same time as the third non-inoculated dish. After formolization, the third dish was inoculated with this same bacterial suspension. All these dishes are incubated at 37° C for 48 hours. The results of this test are outlined in Table II. It appears that the absence of bacterial growth is not due to sterilization, but to the fact that the nutrient media exposed to formol no longer permit the development of bacteria. /201

In a simple diffusion test, the absorption of formol by the medium enabled us to explain this phenomenon. The adjunction

TABLE II

Nutrient medium	Growth in		
	1st Petri dish (control)	2nd Petri dish inoculated before formolization	3rd Petri dish inoculated after formolization
Trypticase Soy Agar (TSA)	+	-	-
Nutrient Agar (NA)	+	-	-
Plate Count Agar (PCA)	+	-	-
Tryptone Blucose Yeast Extract Agar (TGYA)	+	-	-
TSA + histidine	+	-	-
NA + histidine	+	-	-
PCA + histidine	+	-	-
TGYA + histidine	+	-	-
TSA + sodium sulfite	-	-	-
NA + sodium sulfite	-	-	-
PCA + sodium sulfite	-	-	-
TGYA + sodium sulfite	-	-	-

Influence of the nutrient medium on the number of bacteria surviving after formalin evaporation 20 ml/m^3 (Exposure time: 2 hours; temperatures: 18 - 24° C; relative humidity: 65 - 85%)

of formol-inactivating substances such as histidine and sodium sulfite remains ineffective, and even sodium sulfite in itself seems to inhibit bacterial growth.

2.4. As a result of the preceding, we no longer introduced Petri dishes into our evaluative tests. In fact, the scheme henceforth will consist of:

a. the cylinder method, in which microcylinders are inoculated by submersion in suspensions of St. aureus NCTC 4163, E. coli NCTC 8196, and Ps. aeruginosa NCTC 6749;

b. the slide method, in which sterile slides are inoculated with 0.1 ml of bacterial suspension. After exposure to formol, these slides are coated with a thin layer of nutrient medium which is allowed to solidify. The object of this test is twofold. It allows us to determinate the inoculum, and the slides are also easier to sterilize than the cylinders, since the gases can reach them more easily;

c. tests with spores, in which microcylinders and slides are inoculated with B. subtilis spores (wild strain HH or ATCC 19.659). The spores are vacuum dried on $CaCl_2$ according to the A.O.A.C. directives [2] concerning sporicide testing. All the tests are executed in duplicate: half the carriers are placed in the middle of the test room; the other half are placed in a corner.

2.5. A number of methods, currently applied to the terminal decontamination of rooms, were than examined following this scheme. The results of these tests are represented in Table III; only the values applying to Staphylococcus aureus are shown. As related to the use of formol for the terminal decontamination of a room, these results seem deceptive. Yet the formolization methods applied and the formaldehyde concentrations generated meet generally widespread, accepted French and English standards. /202 The conclusion must be that the number of bacteria undergoing formolization was too great in all of our preceding tests. On the basis of the slide method, we were able to calculate, in effect, that inocula bearing one-hundred thousand to two million organisms should have been killed.

This failure proves that formol sterilization will be effective only on small numbers of bacteria; in other words, the room to be disinfected must be cleaned beforehand.

TABLE III

Method applied	Quantity of formalin or paraformaldehyde	Inoculum	Growth			
			Cylinders	Slides	Cylinders	Slides
Formalin-$KMnO_4$	15 ml/m^3	$2.0 \cdot 10^8$	+	-	+	-
Formalin ebullition	15 ml/m^3	$0.8 \cdot 10^8$	+	+	+	+
Formalin nebulization	15 ml/m^3	$1.7 \cdot 10^7$	+		+	
Formalin evaporation	15 ml/m^3	$1.0 \cdot 10^7$	+	+	+	+
Paraformaldehyde sublimation	5 g/m3	$0.8 \cdot 10^8$	+	+	+	+

Effectiveness of different formolization methods on St. aureus (Exposure time: 6 hours)

2.6. On the basis of this finding, we completed the previously outlined scheme by a parallel series in which the inoculum is diluted ten-thousand-fold: the cylinders and the slides are therefore inoculated either with the undiluted bacterial suspension (original tests) or with the diluted suspension.

Furthermore, it follows from the extinction tests that a conclusion based on a single cylinder or slide is valueless, and that is why each test henceforth includes sets of 10 microcylinders and 10 slides inoculated with diluted and undiluted suspensions. The forty objects are placed in a designated corner of the test room.

We finally repeated the previous tests according to this procedure. The object was to examine the different methods generally applied in the formolization of air. The results are reported in Tables IV, V, VI, and VII. In an effort to simplify

TABLE IV

Species of microorganisms	Count of original bacterial suspension	Number of tests showing growth			
		Original suspension		Diluted suspension	
		Cylinders	Slides	Cylinders	Slides
St. aureus	$3.6 \cdot 10^7$	5 of 10	0 of 10	1 of 10	0 of 10
E. coli	$1.3 \cdot 10^8$	0 of 1	0 of 1	0 of 1	0 of 1
Ps. aeruginosa	$1.0 \cdot 10^8$	0 of 1	0 of 1	0 of 1	0 of 1
B. subtilis ATCC		0 of 1	1 of 1		

Evaluation of the terminal decontamination by formalin evaporation: 18 ml/m^3 [Exposure time: 6 hours; temperature: 17 - 24° C; relative humidity: 77 - 60%; maximal HCHO concentration in air: 1.48 mg/l (1206 ppm) (after 60 min)]

the presentation, we only reported on the data for one corner.

They demonstrate that it is possible to sterilize low-density inocula by the current methods, with the exception of trioxymethylene sublimation which takes place without water vaporization: staphylococcus aureus organisms are resistant to this treatment.

TABLE V

Species of microorganisms	Count of original bacterial suspension	Number of tests showing growth			
		Original suspension		Diluted suspension	
		Cylinders	Slides	Cylinders	Slides
St. aureus	$0.7 \cdot 10^8$	10 of 10	8 of 10	3 of 10	0 of 10
E. coli	$1.2 \cdot 10^8$	0 of 1	0 of 1	0 of 1	0 of 1
Ps. aeruginosa	$0.6 \cdot 10^8$	1 of 1	1 of 1	0 of 1	0 of 1
B. subt. ATCC		0 of 1	1 of 1		
B. subt. HH		1 of 1	1 of 1		

Evaluation of terminal decontamination by trioxymethylene sublimation: $5 g/m^3$ [Exposure time: 6 hours; temperature: 21 - 26° C; relative humidity: 73 - 63%; maximal HCHO concentration in air: 1.02 mg/l (831 ppm) (after 75 min)]

TABLE VI

Species of microorganisms	Count of original bacterial suspension	Number of tests showing growth			
		Orig. suspension		Diluted suspension	
		Cylinders	Slides	Cylinders	Slides
St. aureus	$2.0 \cdot 10^8$	9 of 10	4 of 10	0 of 10	0 of 10
E. coli	$1.9 \cdot 10^8$	0 of 1	0 of 1	0 of 1	0 of 1
Ps. aeruginosa	$1.0 \cdot 10^8$	0 of 1	0 of 1	0 of 1	0 of 1
B. subt. ATCC		0 of 1	1 of 1		
B. subt. HH		1 of 1	1 of 1		

Evaluation of terminal decontamination by formalin (15 ml/m^3) -$KMnO_4$ mixture [Exposure time: 6 hours; temperature: 21-26° C; relative humidity: 70 - 63%; maximal HCHO concentration in air: 1.68 mg/l (880 ppm) (after 30 min)]

TABLE VII

Species of microorganisms	Count of original bacterial suspension	Number of tests with growth			
		Original suspension		Diluted suspension	
		Cylinders	Slides	Cylinders	Slides
St. aureus	$0.8 \cdot 10^8$	10 of 10	8 of 10	0 of 10	0 of 10
E. coli	$1.7 \cdot 10^8$	0 of 1	0 of 1	0 of 1	0 of 1
Ps. aeruginosa	$1.2 \cdot 10^8$	1 of 1	0 of 1	0 of 1	0 of 1
B. subt. ATCC		0 of 1			
B. subt. HH		1 of 1			

Evaluation of terminal decontamination by formalin ebullition 15 ml/m^3 [Exposure time: 6 hours; temperature: 20 - 26° C; relative humidity: 73 - 63%; maximal HCHO concentration in air: 1.04 mg/ℓ (850 ppm) (after 15 min)]

3. Discussion

Certain data of the preceding investigations merit a few remarks. /204

3.1. Staphylococcus aureus is an organism which is fairly resistant to formolization. This is demonstrated in Table VIII summarizing 24 tests in which staphylococci, coliform bacilli, and pyocyanic* bacilli were tested at the same time (only the tests on cylinders and in the middle of the test room are listed in the table).

*Translator's note: The group formerly known as Bacillus pyocyaneous is now designated as Pseudomonas group.

TABLE VIII

Degree of resistance of St. aureus	No. of tests	No. of tests with resistance of	
		E. coli	Ps. aeruginosa
Resistant	15	5	4
Sensitive	9	3	2
Totals	24	8	6

It is for this reason that the test using 10 microcylinders and 10 slides is conducted only with staphylococcus.

3.2. Another observation refers to B. subtilis. We simultaneously conducted tests on a cultivated strain (ATCC 19.659) and on a wild strain (HH). Table IX demonstrates that the wild strain is much more resistant to formolization than the ATCC strain. Furthermore, this finding is fairly general; the wild microbe strains are more resistant to antiseptics than the cultivated strains.

3.3. Sporicide testing according to the A.O.A.C. provides for the microcylinder test, but not for the slide test. Believing that the A.O.A.C. tests were too rigorous, we introduced this latter test. We then decided that, on the contrary, in the same investigation, the slides are more often positive than the cylinders. As brought out by Table X, this is true for the B. subtilis spores but not for their vegetative forms.

Moreover, this table emphasizes that without the use of the slide test, the objects in several experiments would have

TABLE IX. COMPARISON OF RESISTANCE TO FORMOLIZATION EXHIBITED BY B. SUBTILIS STRAINS

Degree of resistance		No. of tests
B. subtilis ATCC 19659	B.subtilis HH	
Resistant	Resistant	2
Sensitive	Sensitive	5
Resistant	Sensitive	0
Sensitive	Resistant	9

TABLE X

Species of microorganisms	Cylinders with growth		Cylinders with no growth	
	Slides: growth	Slides: no growth	Slides: growth	Slides: no growth
1. Vegetative organisms				
St. aureus	7	1	0	3
E. coli	1	2	1	7
Ps. aeruginosa	2	1	3	5
Total	10	4	4	15
2. Spores				
B. subtilis ATCC	2	0	8	0
B. subtilis HH	7	0	3	0
Total	9	0	11	0

Comparison of cylinder testing with slide testing

been evaluated as sterile. It therefore seems that the slide method, far from being superfluous, should be retained. /205

3.4. The maximal formol concentration in the air was measured during formolization according to the classic methods [4]. It was impossible to exceed a concentration of 1.7 mg/m^3. This might explain the failure of the tests, since the English directives recommend a formol concentration of 2 mg/m^3. Why did we not attain this presumably active concentration of 2 mg/m^3?

The space concentration of an unstable gas such as formol is a function of three factors: the output of the apparatus, the room temperature, and the disappearance of the gas in the form of inactive polymers which are deposited on the cold surfaces. The manufacturers of formol generators will therefore have to provide devices capable of greater outputs, and there is a need for additional heating of the room being decontaminated. Research should seek to develop substances capable of retarding the so-called polymerizations.

3.5. In our opinion, the investigations demonstrate that formol vapors fail to accomplish the terminal decontamination of rooms. One must therefore seek other chemical sterilizing products which are applicable to the chemical sterilization of air. According to the data from Fort Detrick, where the U. S. Army Biological Center is located, the nebulization of 5.35 ml beta-propiolactone (B.P.L.) would permit one to sterilize a room in several hours [5]. A space concentration of 1 to 1.5 mg beta-propiolactone per liter would be sufficient to kill spores in several minutes at a temperature of 27° C.

Nonetheless, Table XI demonstrates that, judging from our results, the activity of beta-propiolactone is never greater than that of formol. The product concentration in the air did reach a maximal value of 2.26 mg/l (60 minutes after nebulization began), however, and the room temperature was 27° C.

TABLE XI

Species of microorganisms	Count of original bacterial suspension	Number of tests showing growth			
		Original suspension		Diluted suspension	
		Cylinders	Slides	Cylinders	Slides
St. aureaus	$0.8 \cdot 10^8$ b/ml	8 of 10	10 of 10	0 of 10	10 of 10
E. coli	$0.9 \cdot 10^8$ b/ml	1 of 1	0 of 1	0 of 1	0 of 1
Ps. aeruginosa	$3.9 \cdot 10^7$ b/ml	0 of 1	1 of 1	0 of 1	0 of 1
B. subt. ATCC		0 of 1	1 of 1	0 of 1	0 of 1
B. subt. HH		1 of 1	1 of 1	1 of 1	0 of 1

Evaluation of terminal decontamination by cold nebulization of beta-propiolactone 5.3 ml/m^3 [Exposure time: 6 hours; temperature: 26 - 27° C; relative humidity: 80%; maximal B.P.L. concentration in air: 2.26 mg/l (after 60 min)]

3.6.. This poses the problem of the inoculum in a fairly acute manner. Let us remember that by terminal decontamination we mean the sterilization of air and surfaces using a gas. In reality, the decontamination of surfaces can be accomplished by means of liquid decontaminants as well. In this regard, we can cite the directives of the Netherlands (Centraal Instituut voor Voedingsonderzoek) [3], of Great Britain (Colindate Test) [6], and of Germany [1] in which inocula of more than 10^8 organisms per ml are employed.

3.7. We do not wish to close in an atmosphere of hopelessness. Actually, we did not report any tests on the sterilization of air itself. Indeed, the sterilization of air poses no problem. In a series of experiments, we were able to show that the aerosol-borne microbes are easily killed by formol. This signifies that the sterilization of air is easy, but that the sterilization of surfaces is found to be very difficult, if not impossible, especially when these surfaces are heavily infected.

The terminal decontamination of rooms using formol must therefore not be considered as a form of sterilization, but rather as a method of decontamination — that is, a procedure which greatly reduces the number of microorganisms present, but allows certain species, the bacillary spores, for example, to escape.

The terminal decontamination of rooms by formol alone therefore cannot be adequate. The surfaces should be cleaned and disinfected beforehand. The architects should take this into account when choosing materials to cover the walls and floors of sickrooms.

4. Conclusions

4.1. Conclusions Relative to the Method /206

4.1.1. The exposure of inoculated nutrient media as a means of evaluating the room decontamination is without value, since formol exercises a direct inhibitive action on these media.

4.1.2. The evaluation of the effectiveness of surface disinfection must be conducted using inanimate objects: microcylinders, microscope slides or other carriers to be inoculated.

4.1.3. The sensitivity of microorganisms vis-a-vis antiseptics can vary considerably: bacterial spores are more resistant than the vegetative forms; cultivated strains are more sensitive than the so-called wild strains.

4.1.4. The number of organisms present in an inoculum determines whether the disinfectant will be judged active or of no value.

4.2. Conclusions Relative to Formolization

4.2.1. Using available methods, it is not possible to achieve the 2 mg formol concentration per m^3 of air in a room, as recommended by the English authors.

4.2.2. Henceforth, the terminal decontamination of a room cannot be considered as a means of sterilization.

4.2.3. It is necessary to clean and disinfect the surfaces of the room if one wishes to achieve a decontamination which approaches sterility. Air, however, is easy to sterilize.

4.2.4. Formolization cannot yield satisfactory results in a room heavily contaminated with bacillary spores.

4.3. Conclusions Relative to Future Investigation

4.3.1. New investigations are necessary on sporicidal gases.

4.3.2. The size of the inoculum of the different microbe strains being tested must be defined.

4.3.3. Substances retarding the formol polymerization should be perfected.

4.3.4. The minimal space concentration of the disinfecting gases used to decontaminate rooms must be defined.

4.3.5. We think it would be desirable to substitute chemical methods for the biological methods: this would simplify the work and would enable one to have the results in several hours.

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